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Induction of antibody to asialo GM1 by spermatozoa and its occurrence in the sera of homosexual men with the acquired immune deficiency syndrome (AIDS)

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SUMMARY

Compared to healthy homosexual and heterosexual men, homosexual men with acquired immune deficiency syndrome (AIDS) possessed significantly higher levels of IgG antibody to the neutral glycolipid asialo GM1 (ganglio-*N*-tetraosylceramide) ($P < 0{\cdot}01$). Of 31 homosexuals with AIDS, 36% possessed levels of this antibody that were at least two standard deviations above the mean of the healthy men. Furthermore, asialo GM1 antibody could be removed from serum by adsorption with spermatozoa. Weekly rectal insemination of male rabbits with rabbit semen also led to the appearance of antibody to asialo GM1 by 15 weeks. These results suggest that asialo GM1 is a component of ejaculated spermatozoa and demonstrate that rectal insemination by itself can lead to the production of antibodies to this glycolipid in the rabbit. In addition, asialo GM1 antibodies may be of value as a serological marker for the early detection of individuals with AIDS.

Keywords AIDS spermatozoa homosexual men antibodies asialo GM1

INTRODUCTION

An acquired immune deficiency syndrome (AIDS) with a high fatality rate is being diagnosed with increasing frequency among promiscuous homosexual men (Levine, 1982). In addition, AIDS has been reported in Haitians, i.v. drug users and recipients of blood products (Levine, 1982; Menitore *et al.*, 1983).

If AIDS is indeed transferred via blood products, identification of persons in the early or pre-clinical stages of disease is critical for preventing dissemination of AIDS via blood banks. The cause of AIDS remains obscure, although it is generally believed that suppression of the cellular immune response, either by viruses or by other means, is a predisposing factor (Levine, 1982; Sonnabend, Witkin & Purtilo, 1983). We have previously demonstrated that homosexual men have high levels of antibodies to spermatozoa and sperm antigen containing circulating immune complexes (CICs) (Witkin & Sonnabend, 1983). Immune suppression might result from exposure to

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sperm, either by the formation of sperm antibodies cross-reactive with subsets of T lymphocytes (Mathur *et al.*, 1980, 1981), or by the interaction of CICs with IgG Fc receptors on subpopulations of T lymphocytes (Weigle & Berman, 1979). In addition, CIC interaction with Fc receptors induced by cytomegalovirus infection (Keller, Peitchel & Goldman, 1976) may interfere with the function of cytotoxic T lymphocytes. This could explain the alteration in T lymphocyte subset ratios in homosexual men (Kornfield *et al.*, 1982)

An increased susceptibility to infection and malignancies would also result from interference with natural killer (NK) cell function. The intravenous injection of antibody to asialo GM1 (ganglio-*N*-tetraosylceramide), a neutral glycolipid present only on the surface of NK cells (Beck, Gillis & Henney, 1982), has been shown to impair NK function in mice and decrease their ability to reject tumour cells (Kasai *et al.*, 1981). We now report that ejaculated spermatozoa contains asialo GM1 and that antibodies to this glycolipid are found in sera of homosexual men with AIDS and in rectally inseminated male rabbits.

MATERIALS AND METHODS

Subjects. Blood was obtained from 50 homosexual men following a detailed physical examination, from 20 healthy heterosexual men prior to vasectomy, and from 10 additional heterosexual men of proven fertility. Sera were collected by centrifugation, coded and stored in aliquots at −20°C until used. The assays were performed without knowledge of the patients' health status. Thirty-one of the homosexual men were diagnosed as having AIDS, as evidenced by lymphodenopathy (18), Kaposi's sarcoma (five), opportunistic infection (five) or other less defined illnesses (three).

Antibody to asialo GM1. Sera were assayed for antibody to asialo GM1 by an enzyme linked immunosorbent assay (ELISA) modification of a radioimmunoassay (Smolarsky, 1980) and by a newly developed haemagglutination assay using asialo GM1 coated sheep erythrocytes (Dr J. Morgan, Roche Institute of Molecular Biology). For the ELISA, 0·1 ml of asialo GM1 (12 μg/ml in CH_3Cl:Ch_2OH, 3:1) was added to wells of a polyvinyl microtitre plate and evaporated overnight under vacuum. The wells were then washed three times with phosphate-buffered saline containing 0·05% Tween 20 (PBS-Tween) and 0·1 ml of each serum to be tested, diluted 1:10 in PBS-Tween, was added to four wells. Following incubation at room temperature for 120 min, the wells were washed three times with PBS-Tween and 0·1 ml of a 1:200 dilution in PBS-Tween of alkaline phosphatase conjugated swine IgG antibody to human IgG (heavy chain specific, Medical Diagnostics Corp., Hackensack, New Jersey, USA) was added for a second 120 min incubation. The wells were again washed and bound antibody was quantitated as previously described (Witkin & Sonnabend, 1983). Duplicate assay values differed by less than 10% and the mean result is reported. Incubation of sera in wells containing CH_3Cl:CH_2OH without asialo GM1 always yielded a final absorbance of less than 0·05. Binding of asialo GM1 to the wells was confirmed by the use of a rabbit antiserum to asialo GM1.

Absorption with spermatozoa. Duplicate aliquots (0·01 ml) of sera from three men with asialo GM1 antibodies and rabbit antiserum to bovine serum albumin (BSA) were admixed with increasing concentrations of motile human spermatozoa, isolated as described previously (Witkin *et al.*, 1982), in a final volume of 0·28 ml, and incubated for 60 min at 37°C and overnight at 4°C. The spermatozoa were then removed by centrifugation and the sera assayed for IgG antibody to asialo GM1 by ELISA or for antibody to BSA by an ELISA using BSA (1·2 μg/well) fixed to wells of a microtitre plate.

Rectally inseminated rabbits. The procedures and consequences of rectal insemination of male rabbits will be reported in detail elsewhere. Briefly, 1 ml of fresh rabbit semen was deposited 5–6 cm into the rectum of five male rabbits at weekly intervals; 1 ml of buffer was deposited into the rectum of five control males. Sera were assayed for antibodies to asialo GM1 by ELISA using alkaline phosphatase conjugated goat anti-rabbit IgG (heavy and light chain specific, Litton Bionetics, Kensington, Maryland, USA.

RESULTS

Antibody to asialo GM1 in homosexual and heterosexual men

Sera from 19 healthy homosexual men, 31 homosexual men with AIDS and 30 heterosexual men were assayed for IgG antibody to asialo GM1 by ELISA. The results are shown in Fig. 1. If the mean plus two standard deviations of the healthy homosexual and heterosexual values (0·480) is taken as the boundary for normal reactivity with asialo GM1, a total of 11 of the 31 men with AIDS (36%) had an antibody level above this value; 33% (six of 18) of the men with lymphodenopathy, 60% (three of five) with Kaposi's sarcoma, 20% (one of five) with opportunistic infections and 33% (one of three) with other illnesses had increased levels of IgG antibody to asialo GM1. The asialo GM1 antibody level in the homosexuals with AIDS was significantly elevated above the level in healthy men ($P < 0{\cdot}01$).

Six of the sera positive by ELISA for asialo GM1 antibody were also positive by haemagglutination at titres of 1:5 to 1:10. All the other sera were negative in this assay. This confirms the specificity of the ELISA for asialo GM1 antibody and demonstrates its increased sensitivity over the haemagglutination assay.

Effect of spermatozoa on levels of antibody to asialo GM1

To determine if spermatozoa may have been the source of an asialo GM1 immunogen, we measured whether antibody to asialo GM1 was reactive with spermatozoa. Sera from three men with asialo GM1 antibodies were pre-incubated with increasing concentrations of motile human spermatozoa and the effect on asialo GM1 antibody levels was determined. The use of motile spermatozoa minimizes nonspecific binding of IgG to spermatozoa by the Fc region (Witkin *et al.*, 1980). As a further control for non-specific binding, the effect of spermatozoa on levels of BSA antibody was also measured. The results are shown in Fig. 2. The removal of asialo GM1 antibodies from sera was directly proportional to sperm concentrations; BSA antibody levels were unaffected by spermatozoa, demonstrating that the reactivity was specific.

Antibodies to asialo GM1 in rectally inseminated rabbits

To directly determine if the formation of antibody to asialo GM1 is a result of rectal semen deposition, sera from male rabbits who received semen or buffer rectally were assayed for asialo GM1 antibody (Table 1). After 15 weeks of weekly rectal insemination, four of five rabbits demonstrated at least a 50% increase in antibody reactive with asialo GM1; no such increase was evident in the control rabbits. The rabbit asialo GM1 antibody was also removed from serum by

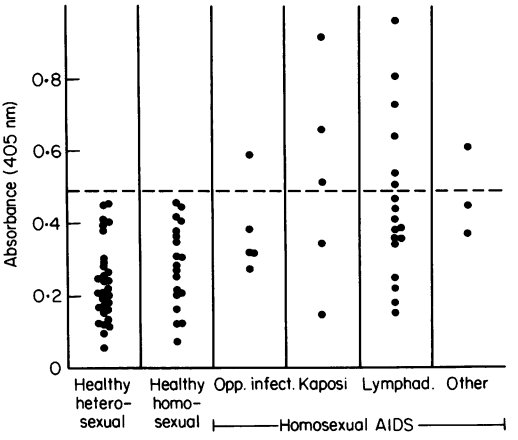

Fig. 1. Antibody to asialo GM1 in homosexual and heterosexual men. Sera from homosexual or heterosexual men were assayed for IgG antibody to asialo GM1 by an ELISA.

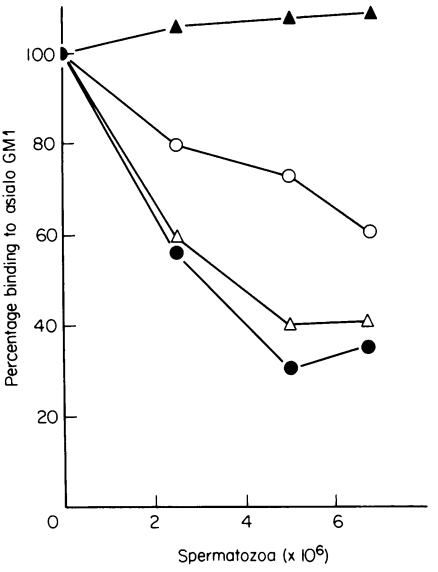

Fig. 2. Effect of spermatozoa on levels of antibody to asialo GM1 in sera of homosexual men. Sera from three homosexual men (●, ○, △) or rabbit antiserum to BSA (▲) were absorbed with spermatozoa and assayed for antibody to asialo GM1 or BSA (▲) by ELISA.

Table 1. Antibody to asialo GM1 in rectally inseminated male rabbits

		Post-treatment/pre-treatment (OD 405)			
Rabbit	Treatment	Week 3	Week 7	Week 9	Week 15
1	Semen	0·99	1·21	1·04	1·30
2	Semen	0·93	0·87	0·79	**2·00**
3	Semen	0·87	0·74	0·92	**2·10**
4	Semen	0·79	1·36	1·46	**1·55**
5	Semen	1·24	**1·95**	**1·92**	**2·12**
1	Buffer	1·06	0·72	0·82	0·91
2	Buffer	1·06	0·98	0·92	1·25
3	Buffer	0·82	0·89	0·68	0·74
4	Buffer	1·08	1·16	1·22	1·22
5	Buffer	0·78	0·73	1·22	1·00

Sera were assayed for antibody to asialo GM1 by an ELISA.

pre-incubation with rabbit sperm (data not shown). This result clearly establishes that rectal semen deposition induces the formation of antibody to asialo GM1.

DISCUSSION

Antibodies to asialo GM1 have been shown to appear as a consequence of rectal deposition of semen in male rabbits. A similar mechanism is probably also responsible for the asialo GM1 antibodies present in homosexual men with AIDS. However, the lack of an association between

antibodies to spermatozoa and to asialo GM1 in men with AIDS, and the presence of sperm antibody (Witkin & Sonnabend, 1983) but not asialo GM1 antibody in sera of healthy homosexual men, suggests that tolerance to asialo GM1 may exist in immunocompetent men. Antibodies to this glycolipid may be produced only in those individuals where normal immune regulatory mechanisms have been altered. In addition, the presence of antibody to asialo GM1 may also reflect an autoimmune response to lymphoid cell components. Asialo GM1 antibody levels are, in fact, inversely correlated with the absolute number and percentage of OKT4 reactive lymphocytes in the circulation of AIDS patients. Preliminary evidence also indicates that women with gynecological malignancies, but not healthy women, have antibodies to asialo GM1.

The evidence that sperm derived asialo GM1 antibody contributes to immune suppression in AIDS or to a specific decrease in NK function is circumstantial. A greatly decreased NK cell activity is frequently observed in men with AIDS (Gerstoft *et al.*, 1982). In mice, NK cell function can be reduced by the intravenous injection of spermatozoa (Hurtenbach & Shearer, 1982) or antibody to asialo GM1 (Beck *et al.*, 1982). In order to provide a more direct link between anti-asialo GM1 antibodies and immune suppression, we are currently trying to identify this glycoprotein on human NK cells, T helper cells, and in factor 8 precipitates.

However, regardless of its ultimate effect on cell-mediated immunity *in vivo*, the correlation of asialo GM1 antibody levels with AIDS suggests that a simple serological assay for the antibody described here may be of value in screening sexually active individuals for disorders of immune regulation, or for screening prospective blood donors.

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REFERENCES

BECK, B.N., GILLIS, S. & HENNEY, C.S. (1982) Display of the neutral glycolipid ganglio-*N*-tetraosylceramide (asialo GM1) on cells of the natural killer and T lineages. *Transplantation*, **33**, 118.

GERSTOFT, J., MALCOW-MØLLER, A., BYGBJEVQ, L., DICKMEISS, E., ENK, C., HALBERG, P., HAAIR, S., JACOBSEN, M., JENSEN, K., MEJER, J., NIELSEN, J., THOMSEN, H., SONDERGAARD, J. & LORENZEN, I. (1982) Severe acquired immunodeficiency in European homosexual men. *Br. Med. J.* **285**, 17.

HURTENBACH, U. & SHEARER, G.M. (1982) Germ cell-induced immune suppression in mice. *J. exp. Med.* **155**, 1719.

KASAI, M., YONEDA, T., HABU, S., MARUYAMA, Y., OKUMURA, K. & TOKUNAGA, T. (1981) *In vivo* effect of anti-asialo GM1 antibody on natural killer activity. *Nature*, **291**, 334.

KELLER, R., PEITCHEL, R. & GOLDMAN, J.N. (1976) An IgG-Fc receptor induced in cytomegalovirus-infected human fibroblasts. *J. Immunol.* **116**, 772.

KORNFELD, H., VARDE STOUNE, R.A., LANGE, M., REDDY, M.M. & GRIECO, M.H. (1982) T-lymphocyte subpopulations in homosexual men. *N. Engl. J. Med.* **307**, 729.

LEVINE, A.S. (1982) The epidemic of acquired immune dysfunction in homosexual men and its sequelae—opportunistic infections, Kaposi's sarcoma, and other malignancies: an update and interpretation. *Cancer Treat. Rep.* **66**, 1391.

MATHUR, S., GOUST, J.M., WILLIAMSON, H.O. & FUDENBERG, H.H. (1980) Antigenic cross-reactivity of sperm and T lymphocytes. *Fertil. Steril.* **34**, 469.

MATHUR, S., GOUST, J.M., WILLIAMSON, H.O. & FUDENBERG, H.H. (1981) Cross-reactivity of sperm and T lymphocyte antigens. *Am. J. Reprod. Immunol.* **1**, 113.

MENITORE, J.E., ASTER, R.H., CASPER, J.T., LAUER, S.J. & GOTTSCHAU, J.L. (1983) T-lymphocyte subpopulation in patients with classic hemophilia treated with cryoprecipitate and lyophilized concentrates. *N. Engl. J. Med.* **308**, 83.

SMOLARSKY, M. (1980) A simple radioimmunoassay to determine binding of antibodies to lipid antigens. *J. Immunol. Meth.* **38**, 85.

SONNABEND, J., WITKIN, S.S. & PURTILO, D.T. (1983) The syndrome of acquired immunodeficiency. An explanation for its occurrence among homosexual men. In *Immune Deficiency and Cancer: Epstein–Barr Virus and Lymphoproliferative Malignancies* (ed. by D.T. Purtilo) Plenum Press, New York (In press).

WEIGLE, W.O. & BERMAN, M.A. (1979) Role of the Fc portion of antibody in immune regulation. In *Cells of Immunoglobulin Synthesis* (ed. by B. Perniss & H.G.Vogel) p. 223. Academic Press.

WITKIN, S.S., SHAHANI, S.K., GUPTA, S., GOOD, R.A. & DAY, N.K. (1980) Demonstration of IgG Fc receptors on spermatozoa and their utilization for the detection of circulating immune complexes in human serum. *Clin. exp. Immunol.* **41**, 441.

WITKIN, S.S., SONNABEND, J. (1983) Immune responses to spermatozoa in homosexual men. *Fertil. Steril.* **39**, 337.

WITKIN, S.S., ZELIKOVSKY, G., BONGIOVANNI, A.M., GOOD, R.A. & DAY, N.K. (1982) IgA antibody to spermatozoa in seminal and prostatic fluids of a subfertile man. *J. Am. Med. Assoc.* **247**, 1014.